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The chromosomes and isoenzymes in marginal populations of the Common shrew (*Sorex araneus*) in the Vistula delta

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Abstract

The chromosomes and isoenzymes of the common shrew populations in the Vistula delta were studied. Twenty nine out of 33 shrews were characterised by the karyotype XX/XY_1Y_2, *af, bc, j/l, hi, g, k, m, n, o, p, q, r, tu*. We suggest that they be described as the Nogat race. The occasional occurrence of *ko* and *gm* metacentrics is the result of introgression from the neighbouring races inhabiting adjacent areas.

Of 22 loci scored, six appeared to be polymorphic. The studied area was homogenous with respect to protein variation. The hypothesis of the presence of a gentle cline of Pgm-3^B allele frequency in the hybrid zone Drużno/Lęgucki Młyn was not supported. The frequencies of esterase (Est-1) and mannosephosphate isomerase (Mpi) fit closely to the pattern of variation for Poland.

Introduction

The karyotype of the common shrew (*Sorex araneus* L., 1758) shows intra- and interpopulation variation based mainly on centric fusions. The species is subdivided into a number of chromosome races, which differ with respect to their metacentric composition or the number of metacentrics (Wójcik 1993; Brünner 1991).

The eastern border of West European Karyological Group (WEKG), according to Hausser et al. (1994), runs through Poland and within its range a few local chromosome races differing only in number of metacentrics were described. Central Poland is inhabited by monomorphic populations of Stobnica race with metacentrics *jl, hi, ko, gm, np* (Wójcik 1993; Fedyk et al. 1993). This monomorphic centre is surrounded by an area of polymorphism for *ko, gm, np* metacentrics and this variation is of clinal nature.

The individual metacentric clines do not coincide. The frequencies of *np* metacentric are the first to decay and the cline of *np* metacentric frequency is the narrowest northwards from the monomorphic centre. The populations characterized by the four remaining metacentrics *jl, hi, ko, gm* were described as Laska race (I) (Wójcik 1986; Fedyk and Leniec 1987; Szałaj et al. 1995). Further north, along the eastern border of WEKG range, the *gm* metacentric frequencies decay gradually and the shrews with metacentrics *jl, hi, ko* were described as Drużno race (Wójcik and Fedyk 1985). East of this area, the frequencies of metacentrics *hi, ko* of Drużno race form narrow clines in the hybrid zone between Drużno and Łęgucki Młyn race (Banaszek 1994). The Łęgucki Młyn race is characterised by metacentrics *jl, hk, io, gr, mn* (Fedyk and Leniec 1987; Wójcik 1993).

In north-western Poland the cline of *gm* metacentric is wider than that of *ko* arm combination. In consequence, the Polish sea coast is inhabited by populations of the Ulm race with the metacentrics *jl, hi, gm* (Wójcik 1986, 1993).

The distance between the closest known sites of Drużno and Ulm races is about 130 km. It is not known whether the range of *ko* and *gm* metacentrics overlap in the area between those points. We have studied chromosomally part of Vistula delta with the aim to describe the local geographic distribution of these metacentrics (Fig. 1). The studies of karyotype variation were supplemented by the electrophoretical analysis.

It is known that genic differences between chromosome races of the common shrew are small (Frykman et al. 1983; Searle 1985; Catzeflis 1984). Clines of allele frequencies between the races were detected only for Mpi locus in northern Sweden (Frykman et al. 1983). Ratkiewicz et al. (1994) suggested also the presence of a gentle cline of the Pgm-3^B allele frequency in the Drużno/Łęgucki Młyn hybrid zone in northern Poland. However the transect studied through the zone was rather short, 18.6 km only. The sample from the Vistula delta can be treated as the westward extension of the transect (Fig. 1) and the hypothesis of the presence of an allele frequency cline can be tested.

Material and methods

Common shrews were collected during 1993 and 1994 from five sites in Żuławy Wielkie. The area is part of Vistula River delta, situated between the Nogat and Vistula Rivers (Fig. 1). The delta was inhabited by shrews rather late, probably in historical times.

The karyotypes of thirty three common shrews were determined (Tab. 1). Chromosome preparations were made in the field by the standard method from spleen (Fedyk 1980). The slides were trypsinized and stained with Giemsa for G-bands (Seabright 1971). Chromosome arms were labelled according to the nomenclature of Searle et al. (1991).

For electrophoretic studies we used 50 individuals from four chromosomally studied sites. The karyotyped shrews were all included in the sample, except for one individual from Dąbrowa. Horizontal

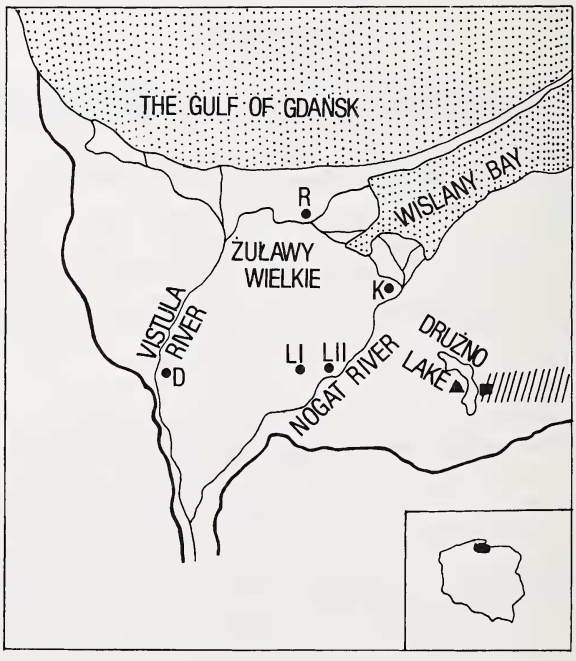

Fig. 1. The distribution of the studied sites; L I – Lubstowo I, L II – Lubstowo II, R – Rybina, K – Kępki, D – Dąbrowa, closed triangle – Krzewsk population, stripped area – the hybrid zone Drużno/Łęgucki Młyn, closed square – Węzina population, thick line – the geographical border of the Vistula delta.

starch electrophoresis of kidney homogenates was carried out as described in Harris and Hopkinson (1976). Buffers and staining procedures were done according to Selander et al. (1971), Harris and Hopkinson (1976) and Quavi and Kit (1980).

Fourteen protein systems coded by 22 presumptive loci were screened. A locus was considered polymorphic when more than one allele was detected in at least one individual. Alleles were designated with letters of Latin alphabet according to the relative mobility of corresponding bands on the gel (Ratkiewicz et al. 1994).

Results

Karyotypes

Individuals with the autosome number 2Na = 26 homozygous with respect to arm combinations *jl, hi* were the most common in all studied populations (Tab. 1, Fig. 2). Three *jl* heterozygotes with 2Na = 27 were also found in Lubstowo I (Tab. 1). The polymorphism of *jl* pair was restricted to that one population only.

Table 1. Material used for chromosome studies. L I – Lubstowo I, L II – Lubstowo II, R – Rybina, K – Kępki, D – Dąbrowa.

Karyotype	2Na	Sample size					
		L I	L II	R	K	D	Total
jl jl, hi hi, – –, – –	26	8	4	6	7	1	26
jl/–, hi hi, – –, – –	27	3	–	–	–	–	3
jl jl, hi hi, ko ko, – –	24	1	–	–	–	–	1
jl jl, hi hi, ko/–, – –	25	–	1	1	–	–	2
jl jl, hi hi, – –, gm/–	25	–	1	–	–	–	1
Total		12	6	7	7	1	33

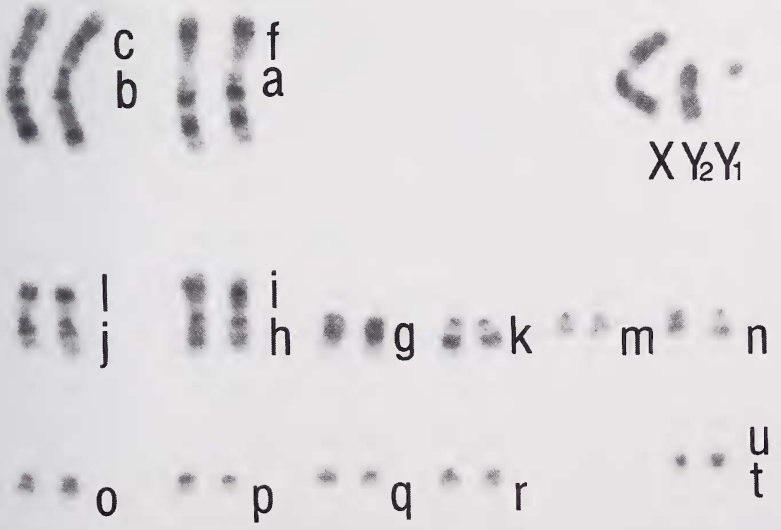

Fig. 2. A karyotype of shrew from the Nogat race.

Metacentric *ko* occurred in three individuals. We found two heterozygotes, one in Lubstowo II and one in Rybina, and one metacentric homozygote in Lubstowo I (Tab. 1). The frequency of metacentric *ko* was 0.08 in Lubstowo I and II and 0.07 in Rybina. One metacentric *gm* was found in Lubstowo II (Tab. 1), which gives the frequency 0.08 of this metacentric in the population. The other chromosome arms of the variable part of the karyotype occurred as acrocentrics in all studied shrews.

Electrophoresis

At the 16 following loci only single alleles were detected: malate dehydrogenase (Mdh-1, Mdh-2) 6-phosphogluconate dehydrogenase (6-Pgd), α-glycerophosphate dehydrogenase (α-Gpd-1, α-Gpd-2), lactate dehydrogenase (Ldh-1, Ldh-2), carbonate dehydrogenase (Est-D), glucosephosphate isomerase (Gpi), superoxide dismutase (Sod-1, Sod-2, Sod-3), glutamate oxalate transaminase (Got-1, Got-2), malic enzyme (Me-2) and isocitrate dehydrogenase (Idh-1).

The six remaining loci appeared to be polymorphic (27.3%): isocitrate dehydrogenase (Idh-2), phosphoglucomutase (Pgm-1, Pgm-3), mannosephosphate isomerase (Mpi), aminoacylase (Acy) and esterase (Est-1). Allelic frequencies at the polymorphic loci are listed in table 2. There were no significant deviations from the Hardy-Weinberg equilibrium in the populations ($P > 0.05$).

At the Idh-2 locus in three of the analyzed sites we found two alleles but only the Idh-2^B allele was detected in Lubstowo II. On the other hand the frequency of the Idh-2^A

Table 2. Allele frequencies at the polymorphic loci in the populations studied and in the pooled sample; in parentheses: number of specimens scored for each locus.

Locus	Allele	Population				Pooled sample
		L I	L II	R	K	
Idh-2	A	0.32(14)	0.00(7)	0.36(14)	0.23(15)	0.26(50)
	B	0.68	1.00	0.64	0.77	0.74
Pgm-1	A	0.00(14)	0.00(7)	0.07(14)	0.00(15)	0.03(50)
	B	0.96	0.93	0.89	1.00	0.94
	C	0.04	0.07	0.04	0.00	0.03
Pgm-3	B	0.75(14)	0.71(7)	0.75(12)	0.89(14)	0.79(48)
	C	0.25	0.29	0.25	0.11	0.21
Mpi*	A	0.00(14)	0.07(7)	0.07(14)	0.00(15)	0.03(50)
	B	0.61	0.79	0.68	0.73	0.69
	C	0.39	0.14	0.21	0.27	0.27
	D	0.00	0.00	0.04	0.00	0.01
Acy	A	0.14(14)	0.14(7)	0.11(14)	0.14(14)	0.13(49)
	B	0.75	0.72	0.85	0.82	0.80
	C	0.11	0.14	0.04	0.04	0.07
Est-1**	A	0.73(13)	0.57(7)	0.61(14)	0.69(16)	0.66(50)
	B	0.27	0.43	0.39	0.31	0.34

* the allele Mpi^A corresponds with the allele Mpi^C in Wójcik and Wójcik (1994), allele Mpi^B with Mpi^A, Mpi^C with Mpi^B. We do not known if the rare allele Mpi^D corresponds with the allele Mpi^D in Wójcik and Wójcik (1994).

** the alleles notations are compatible with those in Wójcik and Wójcik (1994).

allele in the neighbouring population Lubstowo I reached 0.32. Lubstowo II population differs significantly with respect to allelic frequencies at Idh-2 locus from any other population (Fisher's Exact Probability Test $P < 0.05$).

Three alleles were found at the Pgm-1 locus in the studied area. Only one Pgm-1^B allele was detected in Kępki population. It was the common allel in all samples studied. The rare Pgm-1^C allel was also found in all samples except Kępki, while the rare allel Pgm-1^A was detected only in Rybina. Those two rare alleles were only found in a heterozygous state Pgm-1^B/Pgm-1^C, Pgm-1^B/Pgm-1^A.

The other four loci showed polymorphism in all populations studied. Four alleles were recorded at the Mpi locus. The alleles Mpi^B and Mpi^C were detected in all samples and the Mpi^B allel was the commonest. The rare allel Mpi^A was found in two populations Rybina and Lubstowo II, while the Mpi^D was found in Rybina only. Overall five genotypes were recorded: Mpi^B/Mpi^B, Mpi^B/Mpi^C, Mpi^C/Mpi^C, Mpi^B/Mpi^A, Mpi^B/Mpi^D.

We found three alleles at the Acy locus. The Acy^B allele was the commonest in all samples. Two loci Est-1 and Pgm-3 were clearly polymorphic in all populations. Two alleles were detected at both loci.

Discussion

Out of 33 shrews karyotyped, 29 had in their karyotypes *jl* and *hi* metacentrics only. The frequencies of the metacentrics *ko* and *gm* were low and did not exceed the value 0.08 in either population. The shrews with the karyotype XX/XY_1Y_2, *af, bc, j/l, hi, g, k, m, n, o, p, q, r, tu* were thus classified as a distinct chromosome race, which we named Nogat race after the river running close to the sampling sites. The type locality of the race is in Kępki (19°19′ E; 54°12′ N).

The occurrence of *ko* and *gm* metacentrics in the area inhabited by Nogat race is the result of introgression. Metacentrics *ko* were probably derived from the east from the Drużno race. The frequencies of *ko* in Krzewsk population, which is the type locality of Drużno race, reach the value 0.55 (Wójcik and Fedyk 1985), and in Lubstowo I and II situated west of Nogat – 0.08. We do not know the frequencies of these metacentrics on the eastern banks of Nogat. It is probable that the river forms the barrier to chromosome flow and sets the eastern border of Nogat race range. On the other hand the frequency cline of *ko* may be very sharp and the metacentric frequency may be low on both banks of the river. An abrupt decrease in *ko* frequencies was found on the eastern side of Drużno Lake, where the *ko* frequency was 0.33 in the Węzina population (Banaszek 1994) while in the Krzewsk population on the western side of Drużno Lake it reached the value 0.55 (Wójcik and Fedyk 1985).

Metacentric *gm* was found in only one population in the eastern part of the study area and its frequency reached 0.08. This metacentric might have been introgressed not from the west but rather from the southern populations of Laska race along the Vistula River. We do not know the distribution of this metacentric west of Vistula River. We suppose that Vistula forms a strong barrier similar to that of the Nogat River and that this limits the range of Nogat race to Żuławy Wielkie. However, the arm combination *gm* is polymorphic in the populations of Laska and Ulm races in north-western Poland (Wójcik 1986, 1993). It is possible (although less likely) that the cline of *gm* frequencies is narrow in northern Poland and the metacentric does not occur in some areas west of Vistula River. If this holds the occurrence of the shrews of Nogat race would be also expected there.

The populations of Nogat race are situated at the north-eastern edge of the continuous range of WEKG in continental Europe. The number of autosomes in this race ($2Na = 26 – 27$) is the highest yet reported in Poland. These data support the hypothesis

of prevalence of acrocentric chromosomes in marginal populations (Zima et al. 1994). The karyotype of Nogat race is probably ancestral for the WEKG.

It is interesting that shrews with ancestral karyotypes are found in the areas of presumptive pleistocene refugia and also in the areas most distant from them (Zima et al. 1994). It seems plausible that the rate of chromosome evolution was the quickest in the centre of the migration wave. In the refugia, however, as it was suggested by Zima et al. (1994), and also in the forefront of the migration wave, the chromosomal evolution was slow or completely absent. Such hypothesis was also put forward by Wójcik (1993) in his model of chromosomal evolution in the shrews in Central Europe. He argued that centric fusions were formed somewhere within the species range during post-glacial expansion and later extended their ranges.

The electrophoretic study has revealed that Żuławy Wielkie area is rather homogenous with respect to protein variation. The only statistically significant difference was the absence of the Idh-2^B allele in Lubstowo II. However, we treat this difference with great caution as it can be probably attributed to sampling error given the small sample size and the probability of close relations between individuals, since six of them were caught in the same trap.

At the eastern border of Vistula River delta the hybrid zone between Drużno and Łęgucki Młyn race was localized (Banaszek 1994). Six populations from this hybrid zone were studied electrophoretically (Ratkiewicz et al. 1994). The loci Pgm-1 and Idh-2, polymorphic in the present material, were described as monomorphic in this hybrid zone (Ratkiewicz et al. 1994). The description was erroneous, caused by the difficulties in reading zymograms. After the reinterpretation of the data, we found rare heterozygotes at the Pgm-1 locus similarly to this in the present material (Ratkiewicz unpubl. data). We detected also two alleles at the Idh-2 locus in the hybrid zone. The differences between the samples from Żuławy and the hybrid zone in allele frequencies at this locus were not significant (Ratkiewicz, unpubl. data).

We compared the allelic frequencies at the Pgm-3 and Est-1 loci, which were clearly polymorphic in Żuławy and Drużno/Łęgucki Młyn hybrid zone area. We found two alleles at the Pgm-3 locus in the Nogat race sample. Two rare alleles at this locus, which were detected in the shrews of Łęgucki Młyn race, were neither found in Drużno race in the hybrid zone (Ratkiewicz et al. 1994), nor in the Nogat race. Moreover, Ratkiewicz et al. (1994) suggested the presence of the gentle cline for the Pgm-3^B allele frequency through the hybrid zone. The frequency of the Pgm-3^B allele decreased westwards from 0.81 to 0.62. But in the westernmost population the tendency was reversed and the frequency of this allele slightly increased to 0.73 (Ratkiewicz et al. 1994). The extension of the transect westwards to Żuławy, where the Pgm-3 allele frequency was 0.79, confirmed the reversal of the tendency. The hypothesis of the presence of the gentle cline of Pgm-3^B allele frequency in the hybrid zone Drużno/Łęgucki Młyn is therefore not supported by the present data. The slight decrease in the Pgm-3^B allele frequency and corresponding increase in the Pgm-3^C allele frequency can be rather observed in the centre of the hybrid zone.

At the Est-1 locus we found two alleles. Two alleles were also detected in the Drużno/Łęgucki Młyn hybrid zone (Ratkiewicz et al. 1994). The rare Est-1^C allele was detected by Wójcik and Wójcik (1994) in the samples of Białowieża and Drnholec races. But this allele did not occur in the sample of Stobnica race, which is phylogenetically close to the Drużno and Nogat races.

Wójcik and Wójcik (1994) noted that the allele Est-1^A frequency increases westwards in Poland. The frequency of the Est-1^A allele was 0.66 in Nogat race sample. It therefore seems that the frequency of this allele increases in the south-west direction from the lowest values in Białowieża and Nogat race to the maximum in Drnholec race. Slightly higher frequencies of the Est-1^A allele were found in the centre of the Drużno/Łęgucki Młyn

hybrid zone. But it is possible that higher frequencies of the Est-1^A allele were accumulated in the hybrid zone, whereas in the border populations of the transect the frequency was 0.5 on Drużno race side and 0.54 on Łęgucki Młyn race side (Ratkiewicz et al. 1994).

The frequencies of the Mpi^B and Mpi^C alleles form clines ranging from Sweden in the north to Poland in the south (Frykman et al. 1983; Wójcik and Wójcik 1994). The frequencies of Mpi^B – 0.69 and Mpi^C – 0.27 in the total sample from Żuławy fit closely to that pattern of variation. Those frequencies are very similar to the frequencies found in the geographically closest populations of Stobnica race – 0.67 and 0.24, respectively (Wójcik and Wójcik 1994).

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Zusammenfassung

Die Chromosomen und Isoenzyme in Randpopulationen der Waldspitzmaus (Sorex araneus) im Weichsel-Delta

Die Chromosomen und Isoenzyme von Waldspitzmauspopulationen in einem Teil des Vistula-Deltas wurden untersucht. Von insgesamt 33 Spitzmäusen waren 29 durch den Karyotyp XX/XY_1Y_2, *af, bc, j/l, hi, g, k, m, n, o, p, q, r, tu* charakterisiert. Wir schlagen vor, dessen Träger als „Nogat-Rasse" zu bezeichnen. Das gelegentliche Vorkommen von metazentrischen *ko-* und *gm*-Chromosomen ist das Ergebnis einer Introgression von Rassen aus benachbarten Lebensräumen. Von 22 untersuchten Genloci zeigten sechs einen Polymorphismus. Im Hinblick auf die Proteinvariation erwies sich das Untersuchungsgebiet als homogen. Die Hypothese einer geringfügig klinalen Verteilung der Frequenz des Allels Pgm-3^B in der Hybridzone Drużno/Łęgucki Młyn wird durch unsere Daten nicht gestützt. Die Allelfrequenzen an den Loci Est-1 und Mpi stimmen gut mit der generellen Verteilung dieser Allele über Polen überein.

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